# Rapid Detection of Sewer Pipeline Problems Using Bacterial DNA Markers and qPCR Technology

Tao Yan, and Eulyn Pagaling
Dept. of Civil and Environmental Engineering
University of Hawai'i at Manoa

April 10, 2013

Project #: R834871

# OUTLINE

- Introduction/Motivation
- Objectives
- Progress to date
- Ongoing and future works
- Q&A

# BACKGROUND: sewer deterioration

- **Sewer pipe deterioration**
  - Scale: approximately 600,000 miles in U.S.
  - Aging: Average 33 years old.
  - Deterioration: about 75% of sewer infrastructure functioning at <50% design capacity (US EPA 2007).

- **Consequences**
  - Sanitary sewer overflows (SSOs): 23,000-75,000 yearly in U.S. (US EPA 1997).
  - Environmental damage and public health risks
  - Sewer rehabilitation cost in the next 20 yrs: $140 billion to $2 trillion (US EPA 1997).

http://www.lexingtonky.gov/index.aspx?page=2719

# BACKGROUND: Sewer Pipe Problems

- **Common problems**
  - blockages, line breaks, sewer defects, power failure, inadequate design, etc.
- **Causes**
  - Crown corrosion, FOG deposition, root intrusion, dislodged joints…

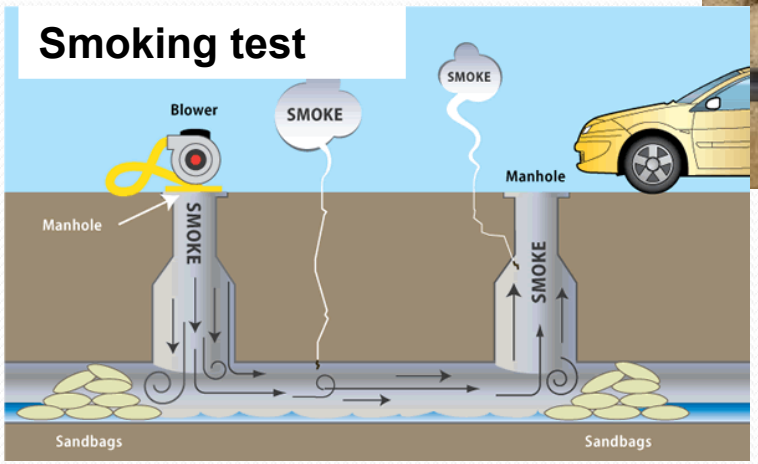


# Current approach

http://urbanaillinois.us/Sanitary_Sewer_System

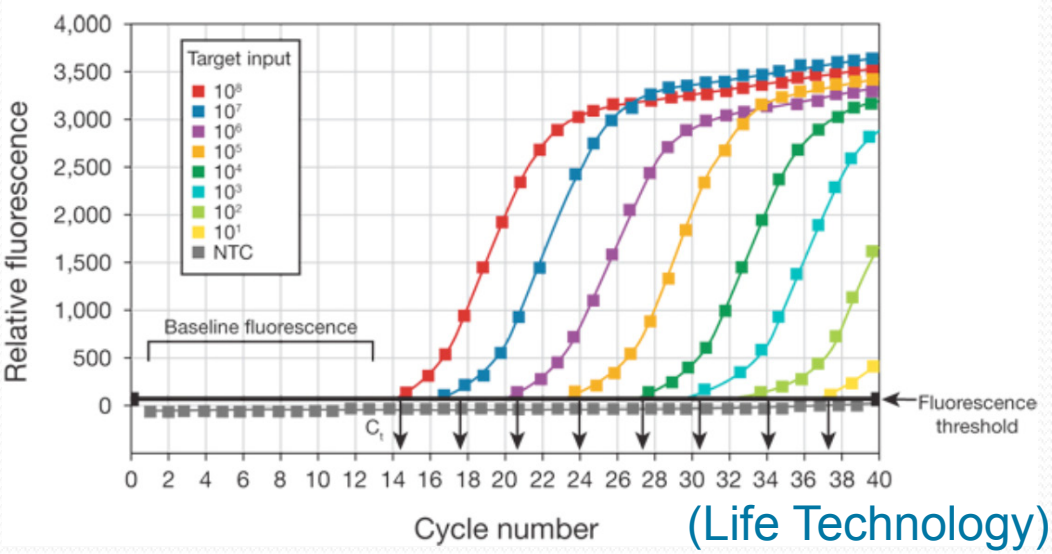
- Reactive and accident-driven
- Better approach: proactive

- Lack of high throughput sewer assessment approach
  - CCTV
  - Smoking test

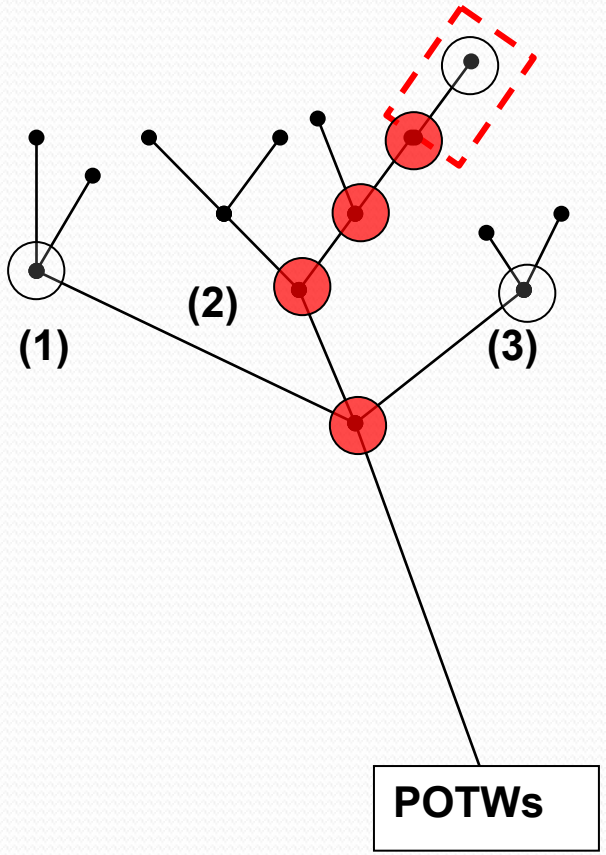



# System-level sewer condition assessment

- **Bacterial DNA markers and qPCR**
  - Sewer pipe problems → Unique bacterial DNA markers
  - Large scale sampling
  - qPCR quantification:



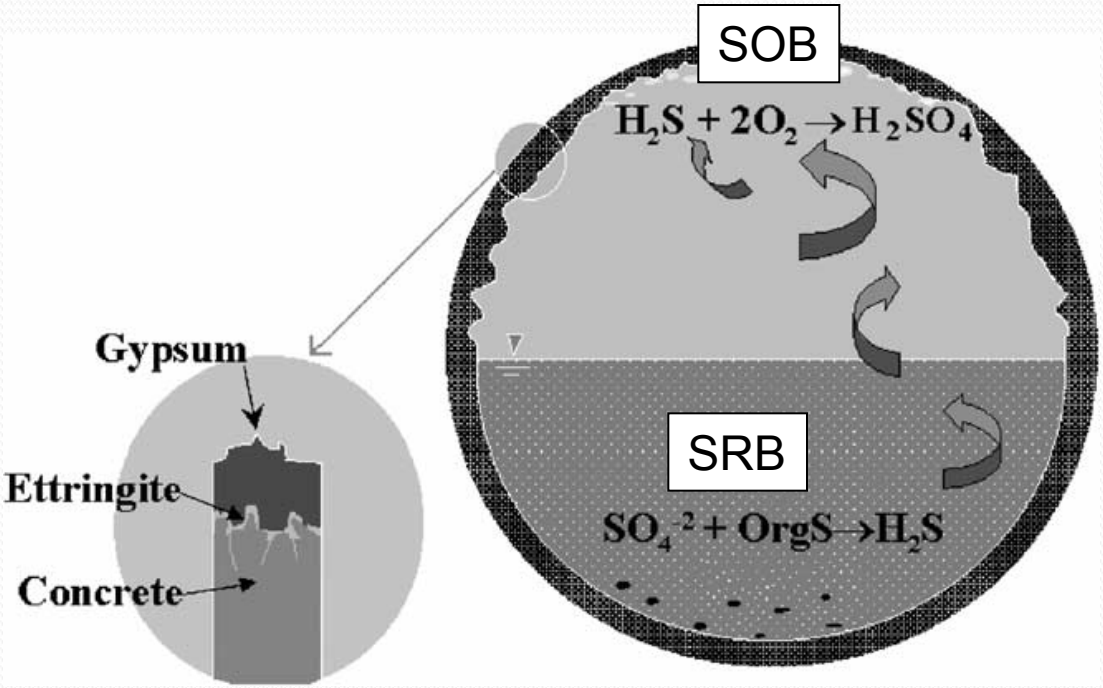

(Life Technology)



# OBJECTIVES

- **Identify bacterial DNA markers for microbially induced sewer crown corrosion (MICC) and FOG deposit.**
- **Develop qPCR assays for quantifying bacterial DNA markers in sewage.**
- **Test the effectiveness of the assays with sewer pipes with known problems**

# MICC




Roberts *et al* (2002)

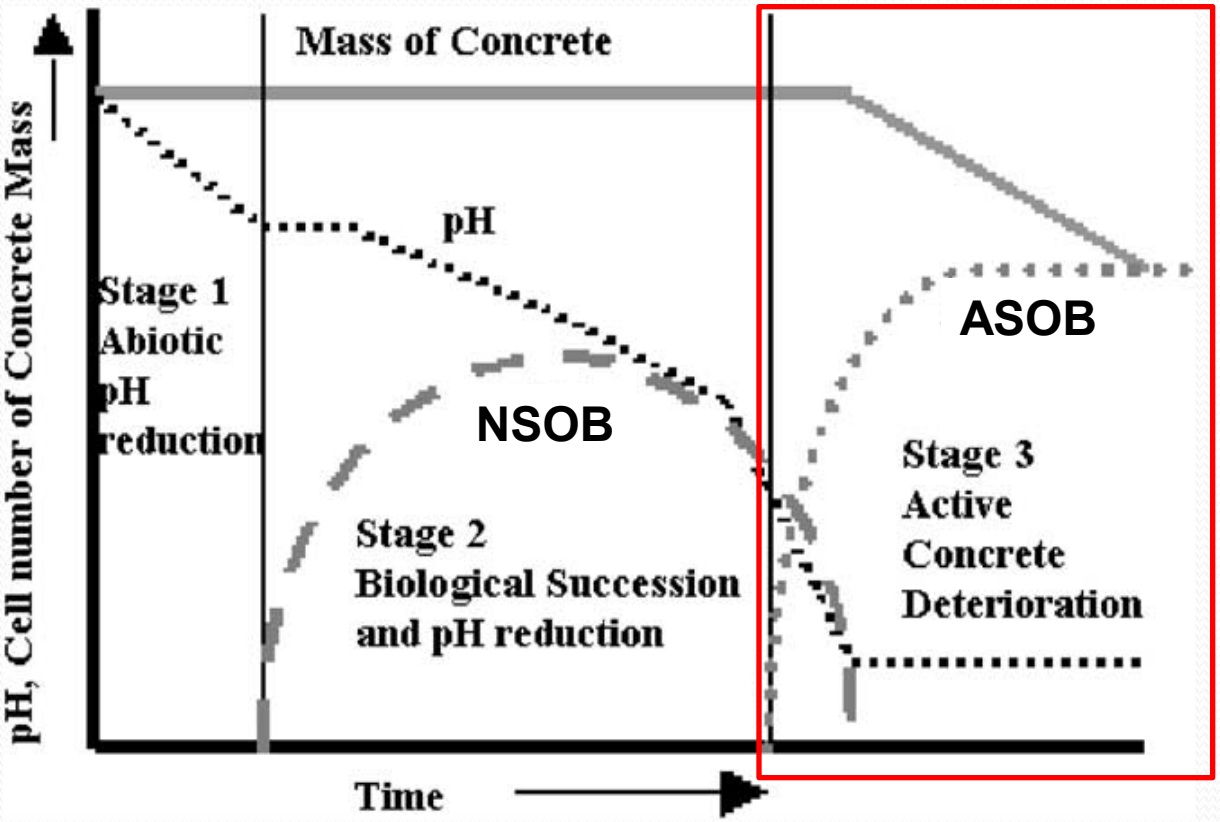
$$2\,H_2O + H^+ + SO_4^{2-} + CaCO_3 \downarrow CaSO_4 \cdot 2\,H_2O + HCO_3-$$

Gypsum

$Ca_6Al_2(SO_4)_3(OH)_{12}\cdot 26H_2O$

Ettringite: calcium aluminium sulfate

# MICC stages and microbes involved




Roberts *et al* (2002).

- Stage 3: active concrete corrosion
- The main ASOB: ***Acidithiobacillus thiooxidans*** (Park 1947, Sand *et al.* 1983, Islander *et al.* 1991)

# *A. thiooxidans* as the marker?

**Molecular survey of concrete sewer biofilm microbial communities**

Biofouling (2011)

Jorge W. Santo Domingo[a]*, Randy P. Revetta[a], Brandon Iker[a], Vicente Gomez-Alvarez[a], Jarissa Garcia[b], John Sullivan[b] and James Weast[b]

[a]*Environmental Protection Agency, Cincinnati, OH, USA;* [b]*Metropolitan Sewer District of Greater Cincinnati, Cincinnati, OH, USA*

(*Received 1 December 2010; final version received 19 August 2011*)

No *A. thiooxidans* detected

E. Vincke · N. Boon · W. Verstraete

**Analysis of the microbial communities on corroded concrete sewer pipes – a case study**

Appl Biotechnol Microbiol (2001)

*Acidithiobacillus, Thiobacillus* and *Mycobacterium*

High-Throughput Amplicon Sequencing Reveals Distinct Communities within a Corroding Concrete Sewer System

AEM 2012

Barry I. Cayford,[a] Paul G. Dennis,[a,b] Jurg Keller,[a] Gene W. Tyson,[a,b] and Philip L. Bond[a]

Advanced Water Management Centre, The University of Queensland, QLD, Australia,[a] and Australian Centre for Ecogenomics, School of Chemistry and Molecular Biosciences, The University of Queensland, QLD, Australia[b]

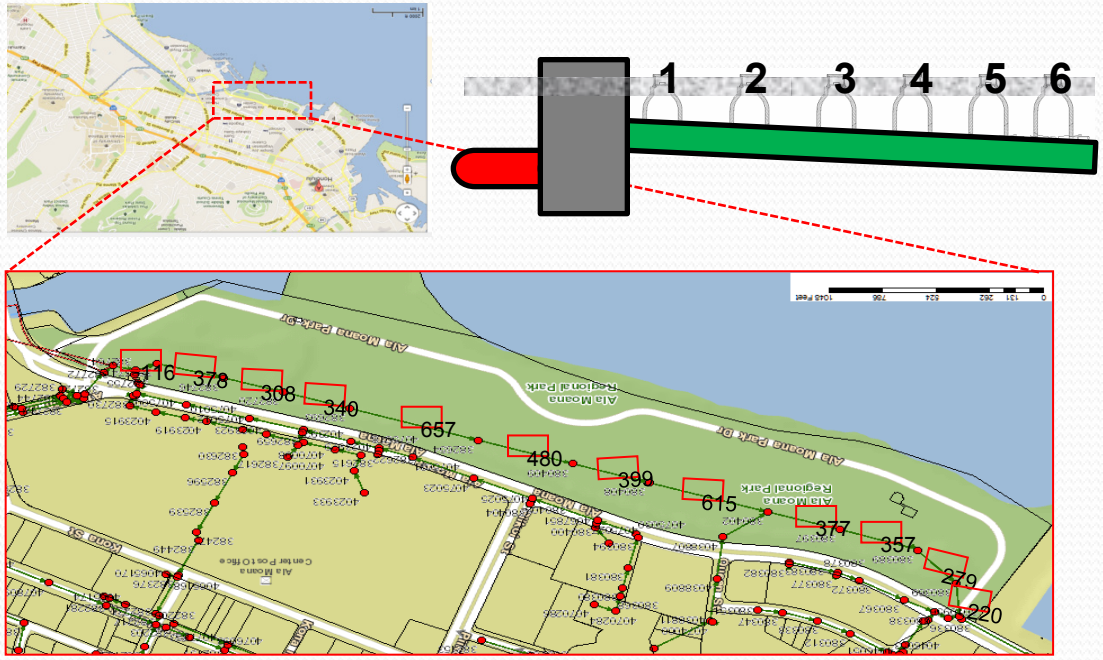
*Acidiphilium* > *Mycobacterium* > *Acidithiobacillus*

75% <3%

# Field sampling

- Questions:
  - Biodiversity of ASOB community?
  - Spatial and temporal variability of ASOB communities?
  - H2S concentration effects?

- Sampling locations
  - Gravity sewer pipes after a forced main.
  - high sewage temp.
  - high to low H2S



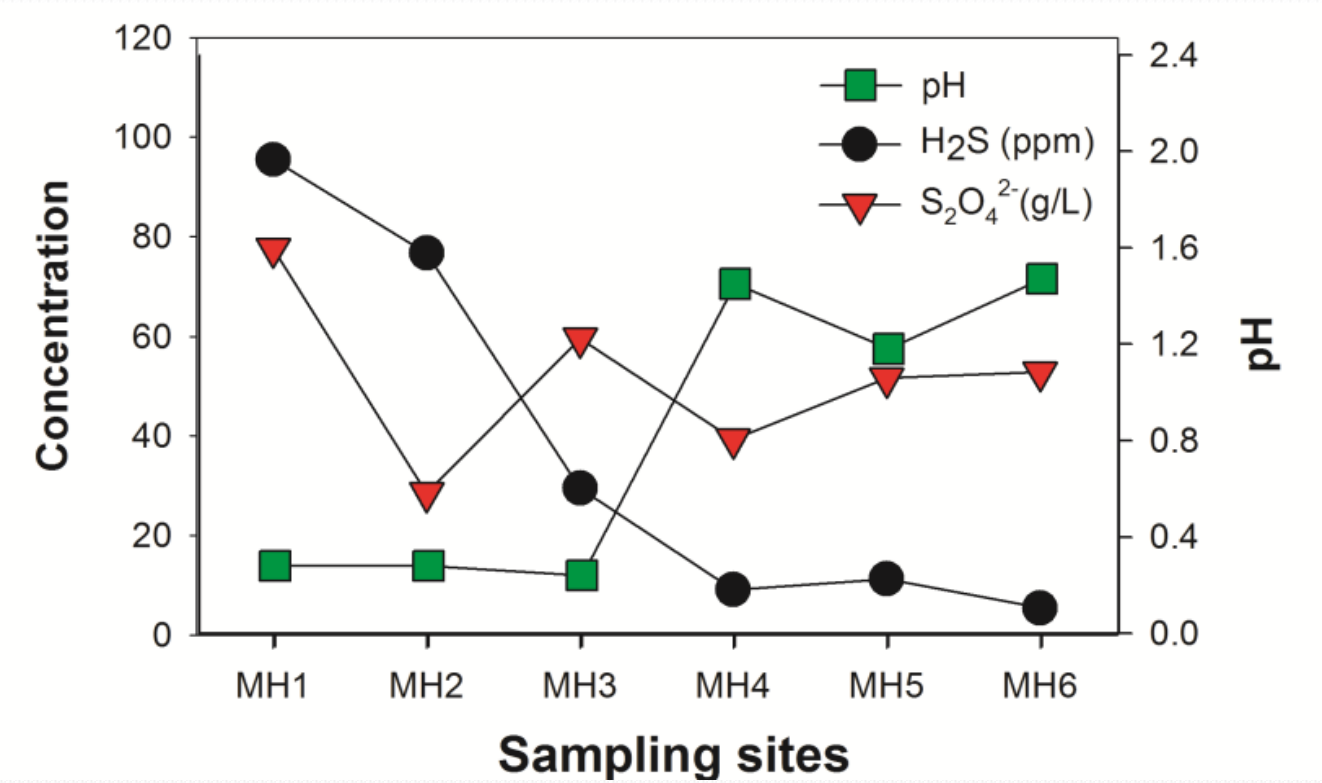
# Field sampling and analysis

- Sampling:
  - Spatial: MH1-MH6,  6/19/2012
  - Temporal: site MH1; 4/2/2012, 6/19/2012, 8/8/2012

- Analysis:
  - In situ measurements of sewer atmosphere $H_2S$.
  - Sewer crown samples analyses:
    - Chemical: $SO_4^{2-}$
    - Mineralogical: X-ray diffraction analysis
    - Bacterial cell density: MPN and qPCR
  - Microbial community analysis
    - 16S rRNA gene pyrosequencing

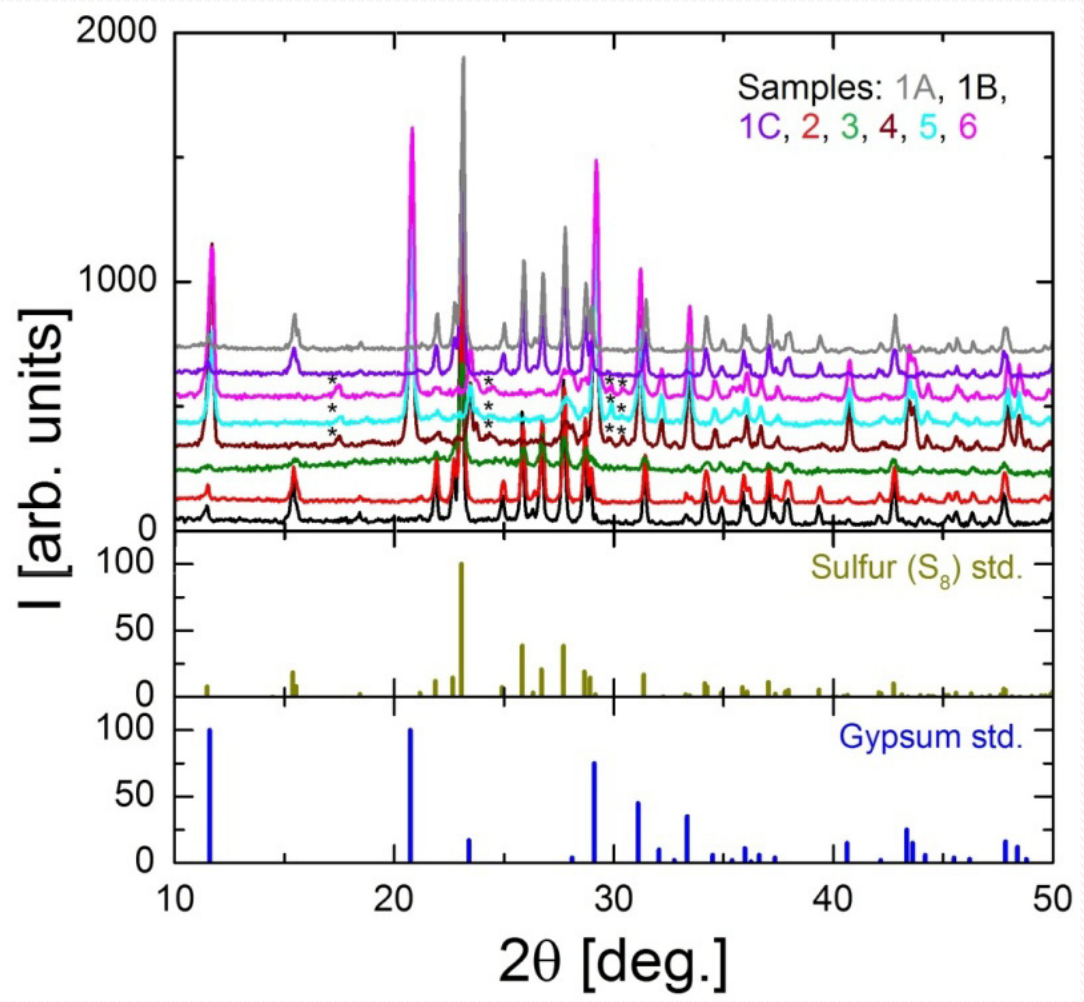
# Crown corrosion at sampling sites

- MH1 → MH6
  - Decreasing $[H_2S]_g$
  - Decreasing $[SO_4^{2-}]$
  - Severe corrosion (all pH< 1.6).



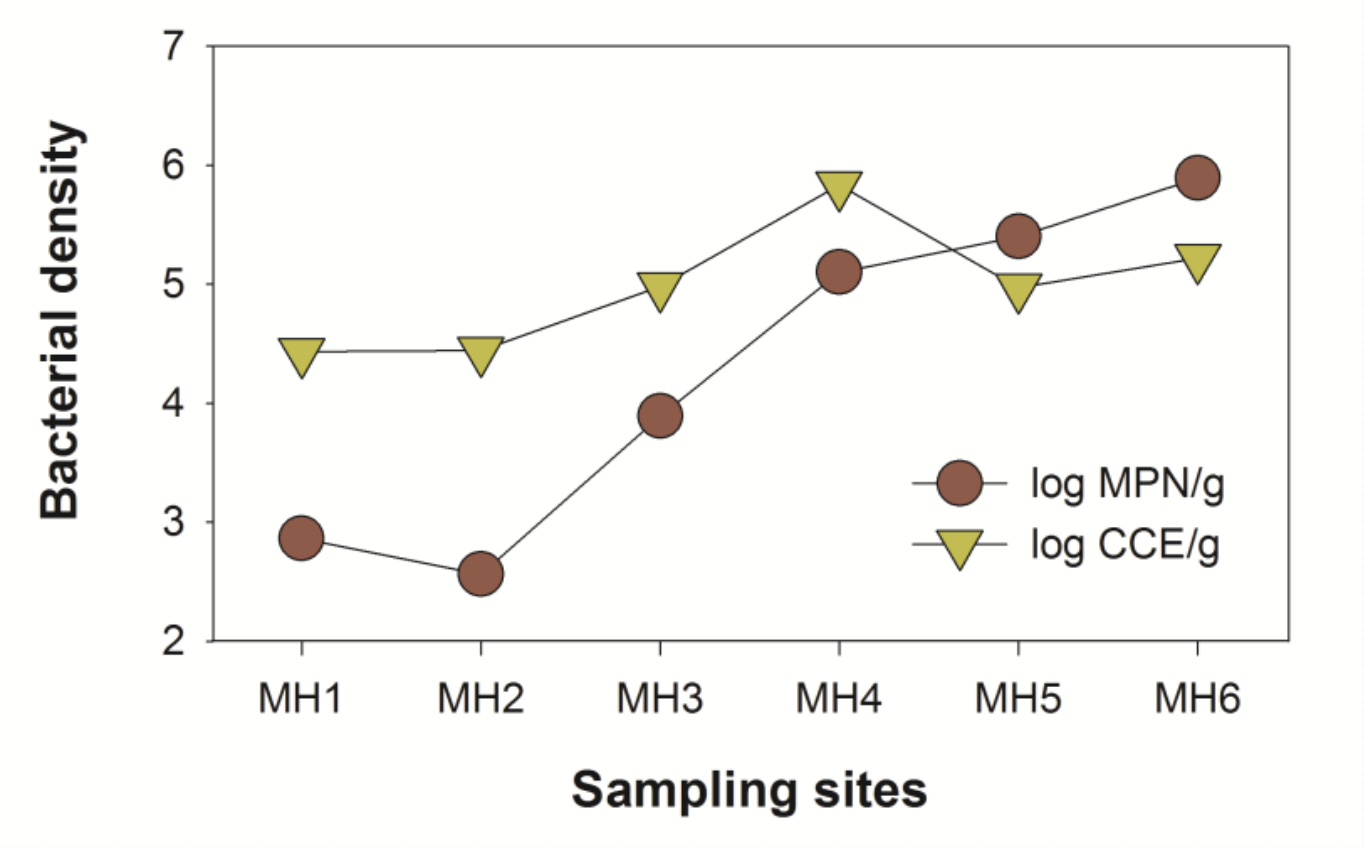
# Crown corrosion products

- MH1-3: elemental sulfur; MH4-6: gypsum

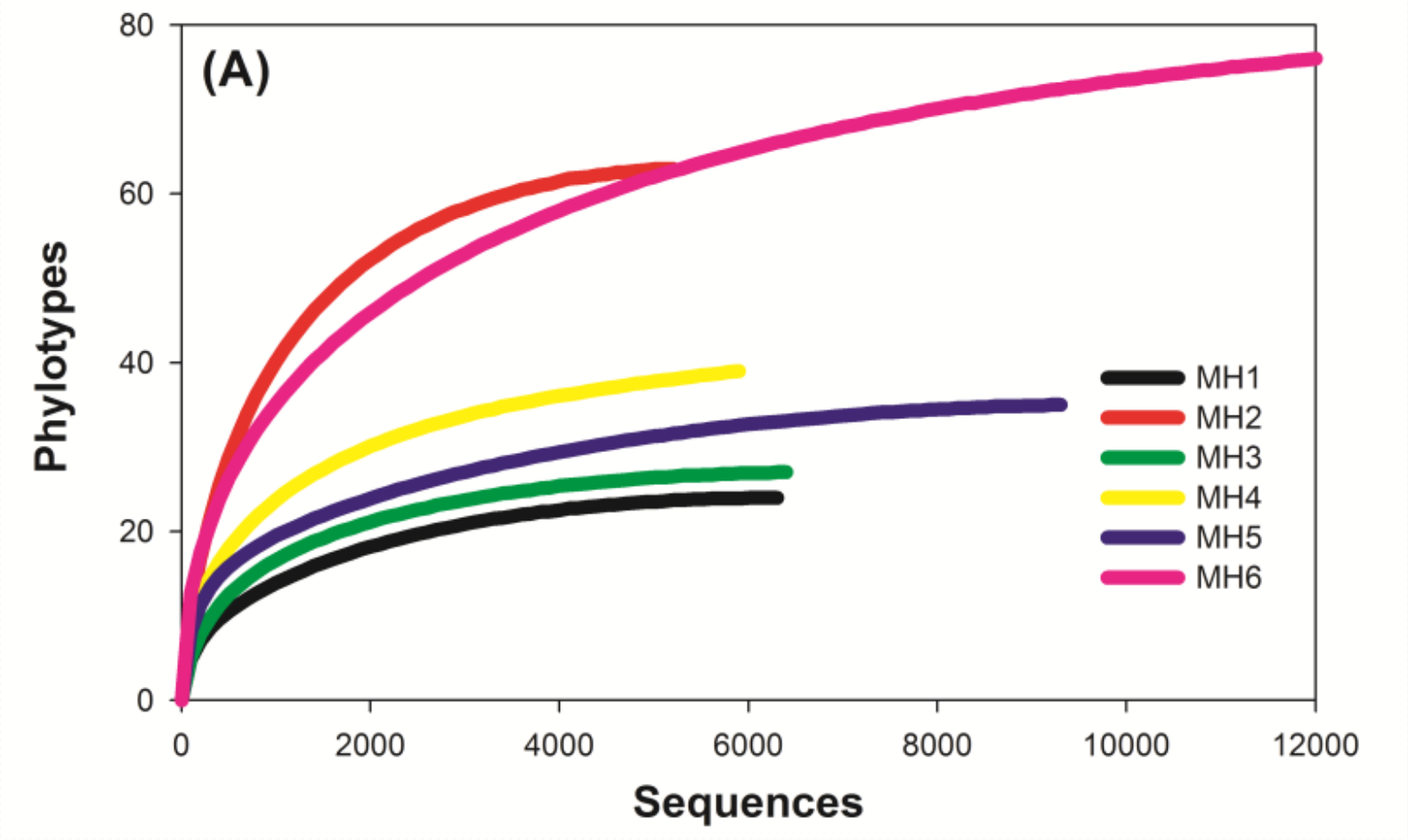


# Total bacterial cell density

- High H2S, low pH (i.e. MH1-3) → lower cell density



# Microbial diversity by pyrosequencing




- 5,000-15,000 sequence reads/sample
- **Majority of diversity recovered**

# Ongoing work

- ***Mycobacterium* sp. as ASOB?**
  - Cultivation sulfur as substrate (using At-OGM (Burlage et al., 1998) and WS5 (Kusumi et al. 2011) ) has only resulted in *Acidithiobacillus* sp.
  - *Mycobacterium*-specific medium (Middlebrook medium) have led to enrichment of *Mycobacterium* sp.
  - Will verify sulfur-oxidation with pure *Mycobacterium* isolates

- Additional field sampling at MH 1-6 planned for the summer

# Future works

- **Conduct field sampling and microbial community analysis to identify bacterial DNA markers for FOG-deposits in sewer pipes**
- **Develop qPCR assays for MICC and FOG deposits in sewer pipes**
- **Conduct field tests to verify the effectiveness of the qPCR assays**

# ACKNOWLEDGEMENT


- Dr. Eulyn Pagaling

- Mr. Loy Kuo of City and County of Honolulu, Hawaii

- Funding: EPA STAR program

# Questions?